Supplementary material

Characterization and quantitation of anthocyanins of the pigmented tea cultivar TRI 2043 (*Camellia sinensis* L.) from Sri Lanka

Philipp Hopfstock [1], P.A. Nimal Punyasiri [2], Mats Kiene [1], J. Dananjava Kottawa-Arachchi [3], Recep Gök [1] and Peter Winterhalter [1]*

[1] Institute of Food Chemistry, Technische Universität Braunschweig, Schleinitzstrasse 20, 38106 Brauschweig, Germany
[2] University of Colombo, Institute of Biochemistry, Molecular Biology and Biotechnology, Colombo 00300, Sri Lanka.
[3] Plant Breeding Division, Tea Research Institute of Sri Lanka, St. Coombs Estate, Talawakelle, Sri Lanka
* Correspondence: p.winterhalter@tu-braunschweig.de

Section S1: MeOH/H_2O extract of TRI2043 (Figure S1).

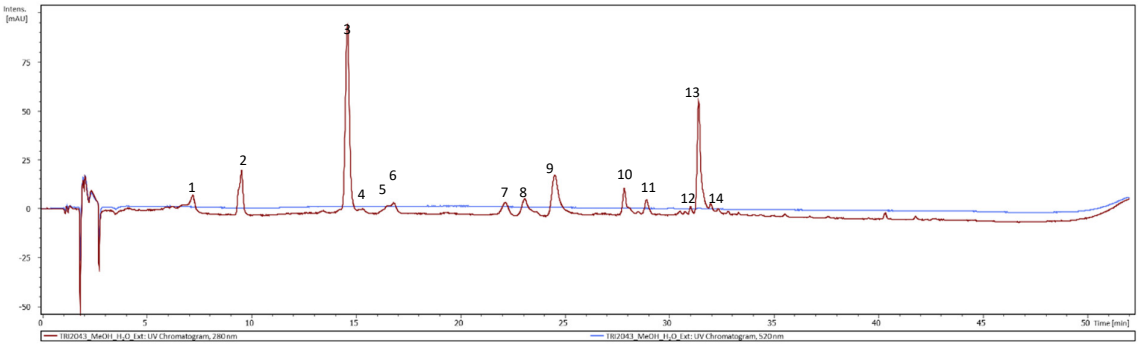

Figure S1. HPLC chromatogram at 280 nm (red line) and 520 nm (blue line) of a MeOH/H_2O TRI2043 extract normalized on the highest peak. The *m/z* of the numbered peaks is shown in table S 1.

Table S1. Summary of HPLC-DAD-MS/MS measurement of a MeOH/H_2O TRI2043 extract (cf. Fig. S 1). Annotation via *m/z* and fragmentation pattern according to the literature [1–3].

Peak #	Compound	$[M-H]^-$	MS/MS
1	5-galloylquinic acid	343	191
2	theobromine	181[a]	na
3	caffeine	195[a]	154/138
4	catechin	289	245/205
5	5-caffeoylquinic acid	353	191
6	strictinin	633	463/301
7	epicatechin	289	245/205
8	4-*p*-cumaroylquinic acid	337	173
9	(epi)gallocatechin-3-gallate	457	331/169
10	(epi)catechin-(epi)gallocatechin	729	559/407/287
11	myricitin-3-*O*-hexoside	479	317/316
12	rutin	609	301
13	(epi)catechin-3-*O*-gallate	441	289/245/193
14	quercetin-hexoside	463	301

[a] $[M+H]^+$

Extract of TRI2043 after enrichment on Amberlite™ XAD7-HP and before membrane chromatography (Figure S2).

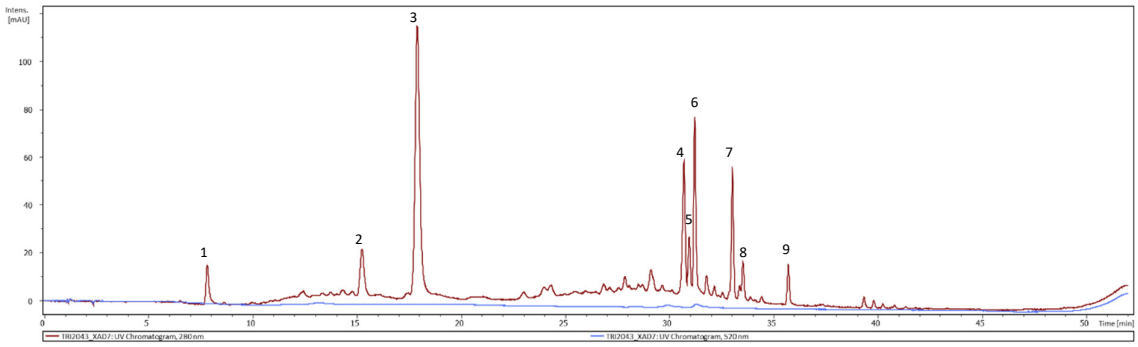

Figure S2. HPLC chromatogram at 280 nm (red line) and 520 nm (blue line) of an Amberlite™ XAD7-HP TRI2043 extract normalized on the highest peak. The *m*/*z* of the numbered peaks is shown in table S 2.

Table S2. Summary of HPLC-DAD-MS/MS measurement of an Amberlite™ XAD7-HP TRI2043 extract (cf. Fig. S 2). Annotation via *m*/*z* and fragmentation pattern according to the literature [1–3].

Peak #	Compound	$[M-H]^-$	MS/MS
1	5-galloylquinic acid	343	191
2	(epi)gallocatechin-(epi)gallocatechingallate	761	609/423/305
3	strictinin	633	463/301
4	quercetin-rhamnose-hexose-rhamnose	755	609/301
5	quercetin-rhamnosylgalactoside/rutin	609	301
6	quercetin-rhamnosylgalactoside/rutin	609	301
7	kaempferol-rhamnose-hexose-rhamnose	739	593/285
8	kaempferol-rutinosid	593	285
9	unknown kaempferol species	781	285

Extract of TRI2043 after membrane chromatography (Figure S3).

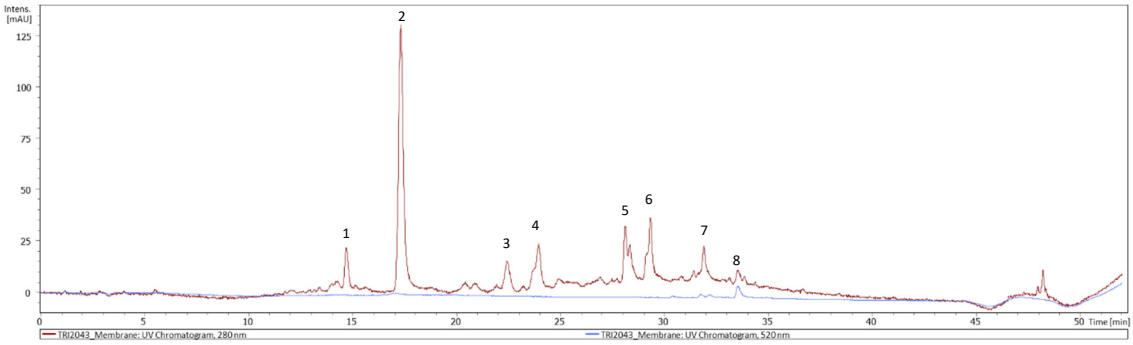

Figure S3. HPLC chromatogram at 280 nm (red line) and 520 nm (blue line) of a membrane chromatography TRI2043 extract normalized on the highest peak. The *m*/*z* of the numbered peaks is shown in table S 3.

Table S3. Summary of HPLC-DAD-MS/MS measurement of a membrane chromatography TRI2043 extract (cf. Fig. S 3). Annotation via *m*/*z* and fragmentation pattern according to the literature [1–3].

Peak #	Compound	$[M-H]^-$	MS/MS
1	(epi)gallocatechin-(epi)gallocatechingallate	761	609/423/305
2	strictinin	633	463/301
3	(epi)catechingallate-(epi)gallocatechin	745	423/305
4	(epi)catechingallate-(epi)gallocatechin	745	407/289
5	(epi)catechin-(epi)gallocatechin	729	407/289
6	strictinin-gallate	785	633/301
7	Rutin	609	301
8	cyanidin-3-*O*-β-D-(6-(*E*)-*p*-oumaroyl)galactopyranoside[a]	595	287

[a] $[M]^+$

HPLC-DAD-MS/MS parameters

pump:	Agilent 1100 G1312A Binary Pump (Waldbronn, D)
autosampler:	Agilent 1200 G1321B ALS SL (Waldbronn, D)
detector:	Agilent 1100 G1315B DAD (Waldbronn, D)
software:	Bruker HyStar V. 3.2 (Bremen, D)
spectrometer:	Bruker HCT Ultra Ion Trap (Bremen, D)
ionization:	electrospray
polarity:	alternating
scan range:	100 – 1500 *m/z*
capillary voltage:	3000 V
nebulizer:	50 psi
dry Gas:	nitrogen 10 L/min
dry Temp:	365 °C
injection volume:	5 µL
column Temp:	25 °C
column:	Luna C18(2) 3µm, 150 mm × 2,1 mm, Phenomenex (Aschaffenburg, D)
flow rate:	0.25 mL/min
solvents:	A: H_2O (Nanopure®, Werner GmbH, Leverkusen, Germany) + 1 % FA (LC-MS grade, Fisher Scientific, Loughborough, U.K.) B: ACN (LC-MS grade, Honeywell Speciality Chemicals, Seelze, Germany)
gradient:	0 min 3 % B, 5 min 10 % B, 15 min 10 % B, 40 min 32.5 % B, 43 min 95 % B, 45 min 95 % B, 45.1 min 3 % B,52 min 3 % B.
wavelength:	λ 200 – 700 nm

Section S2: External calibrations, ranging from 0.5–50 mg/L.

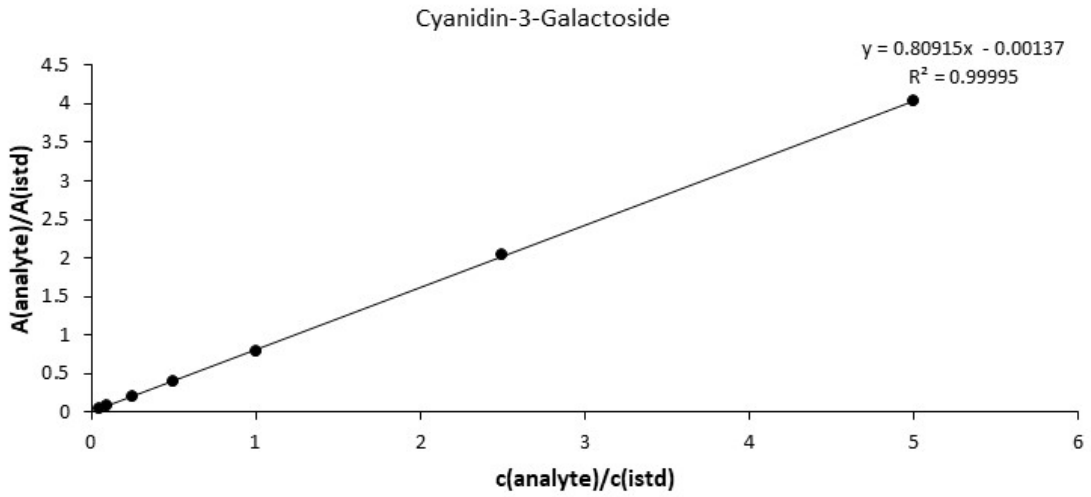

Figure S4. External calibration curve of cyanidin-3-*O*-β-D-galactoside with cyanidin-3-*O*-β-D-glucoside as internal standard c = 10 mg/L.

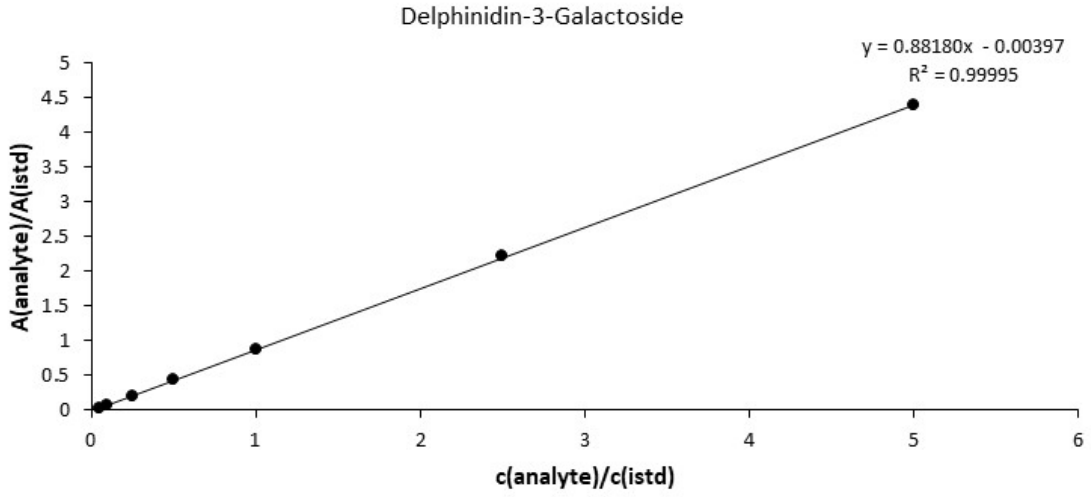

Figure S5. External calibration curve of delphinidin-3-*O*-β-D-galactoside with cyanidin-3-*O*-β-D-glucoside as internal standard c = 10 mg/L.

References

1. Del Rio, D.; Stewart, A.J.; Mullen, W.; Burns, J.; Lean, M.E.J.; Brighenti, F.; Crozier, A. HPLC-MSn analysis of phenolic compounds and purine alkaloids in green and black tea. *J. Agric. Food Chem.* **2004**, *52*, 2807–2815, doi:10.1021/jf0354848.
2. Dou, J.; Lee, V.S.Y.; Tzen, J.T.C.; Lee, M.-R. Identification and comparison of phenolic compounds in the preparation of oolong tea manufactured by semifermentation and drying processes. *J. Agric. Food Chem.* **2007**, *55*, 7462–7468, doi:10.1021/jf0718603.
3. Jiang, X.; Liu, Y.; Li, W.; Zhao, L.; Meng, F.; Wang, Y.; Tan, H.; Yang, H.; Wei, C.; Wan, X.; et al. Tissue-specific, development-dependent phenolic compounds accumulation profile and gene expression pattern in tea plant Camellia sinensis. *PLoS One* **2013**, *8*, e62315, doi:10.1371/journal.pone.0062315.